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RNA binding specificity of hnRNP A1: significance of hnRNP A1 high-affinity binding sites in pre-mRNA splicing.

Burd CG, Dreyfuss G.

Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia 19104-6148.

Pre-mRNA is processed as a large complex of pre-mRNA, snRNPs and pre-mRNA binding proteins (hnRNP proteins). The significance of hnRNP proteins in mRNA biogenesis is likely to be reflected in their RNA binding properties. We have determined the RNA binding specificity of hnRNP A1 and of each of its two RNA binding domains (RBDs), by selection/amplification from pools of random sequence RNA. Unique RNA molecules were selected by hnRNP A1 and each individual RBD, suggesting that the RNA binding specificity of hnRNP A1 is the result of both RBDs acting as a single RNA binding composite. Interestingly, the consensus high-affinity hnRNP A1 binding site, UAGGGA/U, resembles the consensus sequences of vertebrate 5' and 3' splice sites. The highest affinity 'winner' sequence for hnRNP A1 contained a duplication of this sequence separated by two nucleotides, and was bound by hnRNP A1 with an apparent dissociation constant of 1×10^{-9} M. hnRNP A1 also bound other RNA sequences, including pre-mRNA splice sites and an intron-derived sequence, but with reduced affinities, demonstrating that hnRNP A1 binds different RNA sequences with a > 100 -fold range of affinities. These experiments demonstrate that hnRNP A1 is a sequence-specific RNA binding protein. UV light-induced protein-RNA crosslinking in nuclear extracts demonstrated that an oligoribonucleotide containing the A1 winner sequence can be used as a specific affinity reagent for hnRNP A1 and an unidentified 50 kDa protein. We also show that this

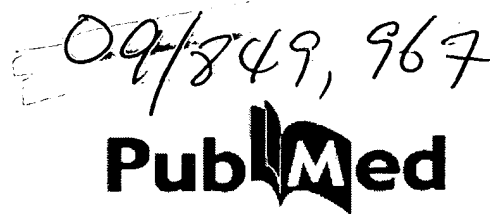
oligoribonucleotide, as well as two others containing 5' and 3' pre-mRNA splice sites, are potent inhibitors of in vitro pre-mRNA splicing.

PMID: 7510636 [PubMed - indexed for MEDLINE]

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